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QUANTITATION OF THE INSECT ELECTRO-ANTENNOGRAM: MEASUREMENT OF SENSILLAR CONTRIBUTIONS, ELIMINATION OF BACKGROUND POTENTIALS, AND RELATIONSHIP TO OLFACTORY SENSATION

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Abstract—A quantitative study of the electroantennogram (EAG) of *Trichoplusia ni* (Hübner) response to sex pheromone has provided new information about the physiological basis of the EAG and its relationship to behaviour. Procedures were developed that selectively stimulated defined numbers of sensilla and eliminated extraneous olfactory stimuli. The results show that the EAG is linearly proportional to the number of antennal sensilla stimulated and clarify interpretation of the blank response. Studies with calibrated dispensers indicate that the EAG is related to pheromone concentration by a power function frequently associated with quantitative measures of electrophysiological and behavioural responses in other animals. Based on correlations between the EAG, behaviour, and single-neurone responses, a model is presented that interrelates these three parameters as different indicators of a single quantitative process in the central nervous system.

Key Word Index: Electroantennogram (EAG), Sex-pheromone, *Trichoplusia ni*

INTRODUCTION

The electroantennogram (EAG) has been used to measure the insect antennal response to stimulation by behaviourally active chemicals since its discovery by Schneider (1957). In the intervening years, however, little effort has been devoted to improving methodology or to understanding its physiological basis. Primarily, the EAG has been applied as a screening tool for identification of sex pheromone components. Occasionally it has been used to predict or interpret behavioural responses. Each of these uses requires the making of several assumptions about the physiological basis of the EAG response. Many of these assumptions are implicit and have not been critically examined.

The objective of this report is to address the most important of the assumptions about the physiological basis of the EAG and to provide a model clarifying its uses for interpreting odourant-mediated behaviour. We present a new methodological sequence that improves the precision and sensitivity of measurements and show that the maximum potential difference induced by a pheromonal stimulus across a *Trichoplusia ni* (Hübner) antenna is correlated statistically with morphometric measurements (Mayer *et al.*, 1981) of the number of pheromone-sensitive sensilla that are stimulated. The regression of maximum potential difference on pheromone concentration is shown to be similar in mathematical form to regressions of behaviour and of single olfactory cell response on pheromone concentration (Mankin and Mayer, 1983). The results support a hypothesis that the EAG, the single-neurone response, and some bioassay responses are all indicators of a central nervous system process that we call sensation.

MATERIALS AND METHODS

Insects and chemicals

Male *T. ni* pupae were obtained from laboratory-reared colonies and the adults were tested 3–5 days after eclosion. The major component of the *T. ni* pheromone, (*Z*)-7-dodecen-1-ol acetate (Z7:12AC), was freed of its (*E*)-isomer by high performance liquid chromatography and further purified by silicic acid column chromatography. Gas–liquid chromatography (GLC) indicated that the refined sample was 99.9 + % pure.

Specimen mounting procedure

Except where otherwise noted intact insects were immobilized inside a glass tube by moulding plasticine around the head, which protruded from the end of the tube. The connection between the amplifier (Grass P-18®) and the preparation was made by inserting several terminal subsegments of one antenna into the tip of an Ag–AgCl pipette electrode filled with 3 M NaCl, sealing the union with a thick, aqueous solution of polyvinylpyrrolidone, and inserting a similar electrode through the contralateral eye.

The restrained live insect was affixed vertically inside Faraday cage enclosures that also reduced stray air currents. Room air was constantly drawn through the enclosures into a fume hood. The dispenser outlet was positioned a few millimeters in front of the antenna at a position where, according

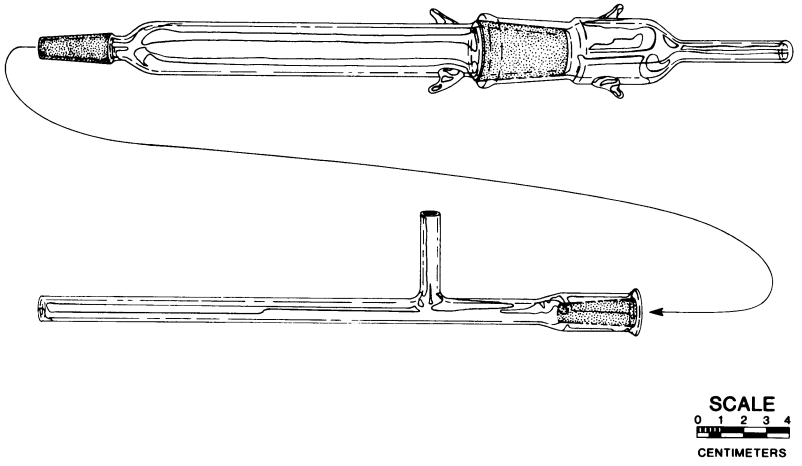

Fig. 1. Diagram of the dispenser and side-arm tube combination developed for quantitative stimulus delivery.

to NH_4Cl smoke visualization, the entire antenna was within the stimulus airstream.

Pheromone dispensers

The dispensers were glass assemblies made by reducing the unground ends of 14 cm long by 2 cm i.d. 24/40 ground-glass joints to 0.5 cm i.d. (Mayer, 1973a). Several of the dispensers were also fitted with a 12/30 ground-glass male joint at one end to accommodate corresponding female joints on side-arm tubes used in some of the tests. The side-arm tube was made from a 22 cm long, 7 mm i.d. glass tube with a 6 mm side arm fused at a right angle 16.5 cm from the outlet end and a 12/30 female ground-glass joint at the inlet end (Fig. 1).

The dispensers were loaded within 4 h of use by coating the inside surfaces with pheromone mixed in a 0.5 ml aliquot of hexane. The hexane evaporated during a short interval of rotation under reduced pressure, and then the dispensers were sealed with cork stoppers. Fifteen to twenty seconds before use stoppers were removed and the dispensers were purged for 30 s with air from a compressed air tank to eliminate room air and reduce the initial nonlinear surge of pheromone release.

Stimulus procedures

The analysis and interpretation of the different EAG responses required the development of several new procedures. The simplest procedure (designated SP1) was used only for stimulus confinement and subsegment resection studies. The outlet end of a loaded and purged dispenser was positioned 1–2 mm upwind of the antenna. The antenna was stimulated by passing a 1 s pulse of dry air from the compressed air tank through the dispenser at 200 ml/min, eliciting a potential difference between the antennal base and tip (antennal polarization). The maximum antennal polarization recorded during stimulation is called the electroantennogram (EAG).

The second method of stimulation (designated SP2) utilized the side-arm tube with a side and a front inlet. The side inlet was connected to a constant flow of filtered, compressed air of either 140 or 1200 ml/min that isolated the antenna from laboratory air. The air that passed through the dispenser into the front inlet of the sidearm tube was maintained at 50 or 200 ml/min by a separate control. All connections and couplings in the system were of either Teflon® or glass.

The procedure for stimulating the antenna by the SP2 method was: (1) initiate the carrier air at the side inlet, noting the removal of room contaminants by the appearance of a positive antennal polarization; (2a) (for low concentrations) enter a time mark on the recorder, 5 s after which a valve to the dispenser was opened, dispensing the stimulus into the front inlet for 5–10 s at the 50 ml/min flow rate and 2 s at the 200 ml/min flow rate; (2b) (for high concentrations) dispense the stimuli for 2 s; (3) record the antennal polarization; and (4) terminate the carrier flow. By following the sequence of steps described here, a "blank" response is avoided.

When concentrations of pheromone were dispensed at or above doses of about 10 μg, small but measurable EAGs occurred in subsequent tests of clean dispensers due to contamination in the side-arm tube. In practice the side-arm tube was changed after any chemical was dispensed at or above 10 μg doses. Generally, the side-arm tubes can be decontaminated by heating them above 170°C for a few minutes.

Analysis of dose–response data

The EAGs were analyzed by two different methods, the power function-analysis introduced here and the more common log-linear analysis. The most widely used method for analysis of EAGs is the fit to the log-linear equation:

$$R = b_1 + b_2 \log D, \qquad (1)$$

where D (μg) is the pheromone dose, b_1 and b_2 are regression coefficients, and R ($-$mv) is the antennal polarization (EAG). Other fits to the data are possible and, from the viewpoint of sensory physiology, other fits may be more useful. For example, although until now there was no direct evidence, it has been commonly assumed that the EAG is correlated to the summed generator potentials from olfactory receptor neurones (Schneider, 1962). Because the responses of the generator potentials probably are power func-

tions of the stimuli (Mankin and Mayer, 1983; Stevens, 1975), it follows that the EAG should fit a power function:

$$R = b_3(C)^b, \quad (2)$$

where C (μmol/cm^3) is the odourant concentration, b_3 and b are regression coefficients, and R ($-$mv) is as before. To determine whether the data fit this type of equation it was first necessary to calibrate the dispenser emission rate at different doses and then determine the pheromone concentration in the stimulus air. The calibration will be described in a later report (Mayer *et al.*, submitted to *J. Chem. Ecol.*).

Stimulus confinement and subsegmental resection tests

Usually the entire antenna was exposed to the stimulus, but in tests to investigate the relationship between the antennal polarization and the number of sensilla stimulated, the stimulus was restricted to specific parts of the antenna. For the stimulus confinement procedure, we modified the dispenser by coupling the tip to a Pasteur pipette with a Teflon sleeve. Smoke visualization showed that the capillary outlet, 1 mm in diameter, dispensed pheromone in a well-defined plume of about the same diameter. The outlet was centred at subsegment 5 from the base and then at each 10th subsegment thereafter, continuing usually to subsegment 60. The head and the recording electrode obstructed the airflow at the antennal extremities. For this procedure and the one following, a single pheromone dose of 10 μg was used.

The second procedure for stimulating different numbers of sensilla was accomplished by progressively resecting subsegments and recording the antennal polarization from the subsegments remaining after each resection. First, several terminal subsegments were resected and the EAG was recorded. Then the ten most distal subsegments were resected and the polarization recorded again. Distal subsegments were removed and the EAG was recorded until too few subsegments remained from which to record.

Analysis of polarization vs number of sensilla stimulated

The results of the stimulus confinement and the resection studies were analyzed by least-square regression analysis according to the equation:

$$R = b_4 + b_5 n, \quad (3)$$

where b_4 and b_5 are regression coefficients, n is the number of sensilla stimulated by the pheromone, and R ($-$mv) is the EAG. To determine the number of sensilla stimulated, we referred to the morphometric study of the *T. ni* antenna by Mayer *et al.* (1981). Table 1 of their study lists empirical equations that estimate the total number of sensilla and the proportion of different types of sensilla on each antennal subsegment as well as the length of each subsegment. The total number of sensilla on subsegment x, where x is the number of subsegments distal to the basal subsegment is:

$$N_x = -34.60725556 + 23.83318776x - 0.93729626x^2 + 0.01401436x^3 - 0.00007389x^4. \quad (4)$$

The proportion of this number that is type I with pheromone-sensitive neurones is:

$$P_x = 1.04415507 - 0.02575705x + 0.00051138x^2 - 0.00000350x^3. \quad (5)$$

Consequently, the total number of type I sensilla between any 2 antennal subsegments, i and j, is the sum:

$$n = \sum_{x=i}^{j} P_x N_x, \quad (6)$$

where i is the most proximal and j is the most distal subsegment.

Two groups of receptor cells having different response thresholds to pheromone have been identified on the antenna of *T. ni*. Both groups are located within sensilla trichodea, previously classified as a single Type (I), which are morphologically distinct at high magnification ($\sim$23,000$\times$) with scanning electron microscopy (Mayer and Mankin, 1984; O'Connell *et al.*, 1983). The presence of these groups complicates the assessment of sensillar contributions to the EAG. We will not make a distinction between the two response groups and their separate contributions in this report.

For each of the stimulus confinement tests the starting and ending subsegment numbers, i and j, were determined from the diameter of the dispenser outlet (1 mm) and the approximate number of subsegments subtended by the dispenser diameter as follows. First, the length of the subsegment at the centre of the stimulated region was calculated from the equation:

$$L_x = 141.3668 + 0.8017x - 0.0194x^2 \quad (7)$$

where x is the subsegment at the center of stimulation and L (μm) is the length of subsegment x. If this length was less than 490 μm, slightly less than the radius of the dispenser, the length of the $x + l$ subsegment was calculated and added to L_x. The process was continued until a subsegment, j, was added that made the total length $L_x + L_{x+l} \ldots + L_j$, greater than 490 μm. By setting $i = 2x - j$ in Eq. **6**, we thus calculated the number of pheromone-sensitive sensilla along a 1-mm section of the antenna from subsegments i to j.

For each of the antennal resection tests, the initial subsegment, i in Eq. **6**, was set equal to 1. The ending subsegment, j, was determined by counting the number of subsegments remaining after the final resection, and then adding 10 subsegments per resection.

RESULTS

The major findings of the EAG measurements can be summarized as follows: (1) the stimulus confinement and antennal resection experiments both yielded statistically significant fits for regressions of EAG on the number of Type I sensilla stimulated; and (2) the stimulus–response curve for Z7:12AC is a statistically significant fit to a power function. Of further significance from the methodological comparisons was that the new stimulation procedure developed for this study significantly enhances the precision and the sensitivity of the EAG technique.

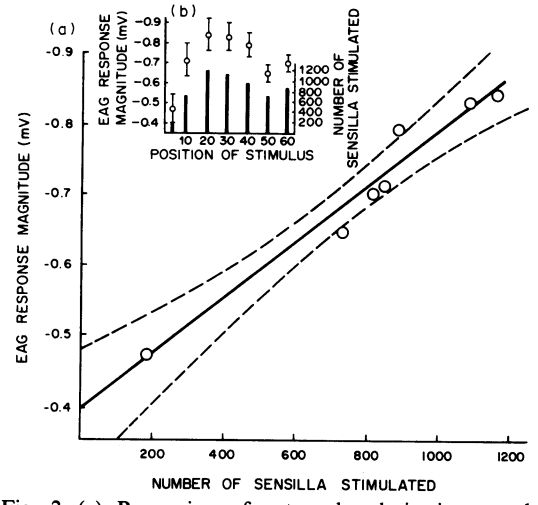

Fig. 2. (a) Regression of antennal polarization on the number of sensilla trichodea (Type I) subtended by confined stimulus ($C = 4 \times 10^{-5}\,\mu g/cm^3$; avg. of 5 antennae). (b) The mean responses ± standard errors at particular positions on the flagellum (circles, left ordinate) and the number of sensilla trichodea (Type I) at the stimulus site (bars, right ordinate).

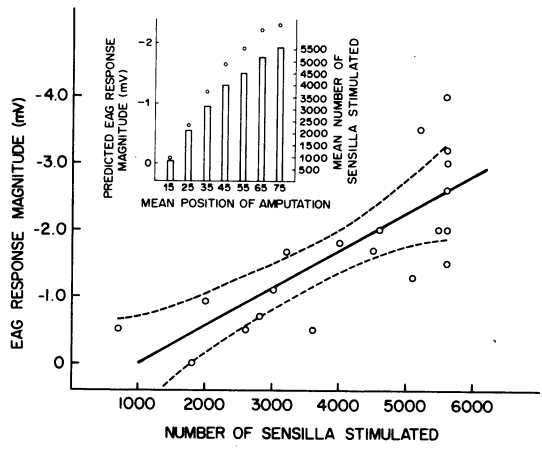

Fig. 3. Regression of antennal polarization on the number of stimulated sensilla trichodea (Type I) remaining on progressively resected antennae ($C = 4 \times 10^{-5}\,\mu g/cm^3$; avg. of 5 antennae). Inset: mean responses from antennae resected at the designated position (circles, left ordinate) and the total number of sensilla trichodea (Type I) on the remaining flagellar subsegments (bars, right ordinate).

The physiological basis of the EAG

The stimulus confinement procedure yielded the EAG responses shown in Fig. 2a–b. The relationship of elicited responses to the number of Type I sensilla trichodea, estimated from Eq. **6**, is shown in Fig. 2a. The line of best fit is

$$R = 0.425 + 5.32 \times 10^{-4} n \qquad \textbf{(8)}$$

where R ($-$mv) is the EAG and n is the number of sensilla stimulated. The standard errors are 0.068 and 1.16×10^{-4} for the intercept and slope, respectively, and the coefficient of determination is $r^2 = 0.81$.

The subsegment resection tests were done first with the SP1 procedure and were later confirmed using SP2. In the SP1 experiments the response was proportional to the number of antennal subsegments remaining (Fig. 3b). The regression of the antennal polarization on the number of stimulated Type I sensilla trichodea is shown in Fig. 3a. The line of best fit is

$$R = -0.458 + 4.02 \times 10^{-4} n \qquad \textbf{(9)}$$

with the terms defined as in Eq. **8**. The standard errors are 0.263 and 6.5×10^{-5} for the intercept and the slope, respectively, and the coefficient of determination is $r^2 = 0.86$.

The second series of antennal resection experiments was performed with SP2 to test the stimulus procedure and further to confirm the previous resection measurements. For the second series the regression of EAG on the number of Type I sensilla trichodea yielded

$$R = -0.441 + 5.10 \times 10^{-4} n \qquad \textbf{(10)}$$

with the terms defined as in Eq. **8**. The standard errors were 0.409 and 9.49×10^{-6} for the intercept and the slope, respectively, and the coefficient of determination was $r^2 = 0.60$. Thus, both resection and stimulus confinement procedures estimate the mean contribution to the EAG from each Type I sensillum to be approximately 0.5 μv at a dose of 10 μg.

Stimulus–response relationship

The mean EAG elicited at different levels of Z7:12AC is shown in Fig. 4a as a regression on log concentration and in Fig. 4b as a regression on log

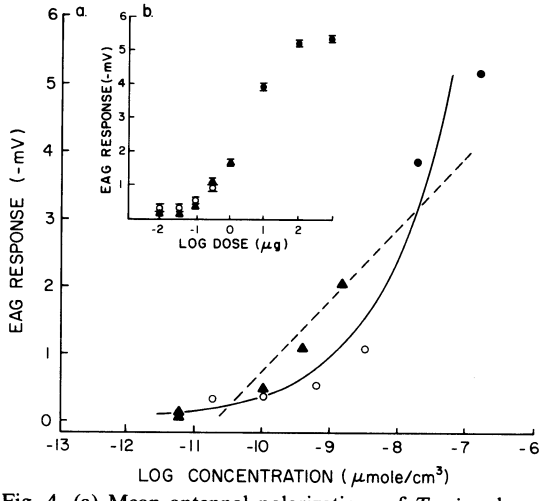

Fig. 4. (a) Mean antennal polarizations of *T. ni* males at different concentrations of Z7:12AC. (b) Mean antennal polarizations at different dispenser doses. Note that the releases rates of the 10^2 and 10^3 μg doses in (b) were equal so that they merge in (a). Symbols: open circle, mean responses of 19 males obtained at dispenser flow of 50 ml/min and carrier flow of 140 ml/min; filled circles and triangles, mean responses of 15 males obtained at dispenser flow of 200 ml/min and carrier flow of 1200 ml/min (the different symbols indicate that different calibration equations were applied in converting a dose level of Fig. 4b into an equivalent concentration level of Fig. 4a) solid line, power function of best fit; dashed line, log-linear function of best fit.

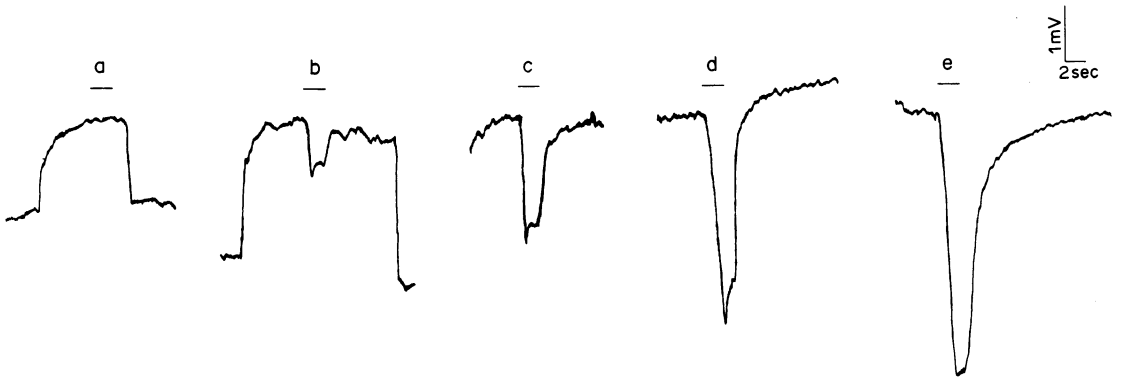

Fig. 5. Examples of antennal polarizations obtained with Z7:12Ac by the SP2 method. Solid bar denotes stimulus; air flow = 200 ml/min; stimulus concentrations (μg/cm^3); a = 0; b = 1.1×10^{-5}; c = 1.2×10^{-4}; d = 1.2×10^{-3}; e = 1.2×10^{-2}.

dose. The concentration data in Fig. 4a were calculated from equivalent dose data in Fig. 4b by applying three different dispenser calibration equations described in a separate report submitted to *J. Chem. Ecol.* The results in Fig. 4 demonstrate the utility of describing the EAG in terms of odourant concentration rather than dispenser dose. Without expressing the stimulus in terms of concentration, it would not be possible to combine meaningfully the responses from the two different stimulation procedures.

A power function regression equation fits EAG response data as well as or better than a log-linear function (Fig. 4) (Mayer, 1973b). The power function coefficient estimates (see Eq. **2**) are $\log b_3 = 1.94 \pm 0.21$ and $b = 0.22 \pm 0.02$. The log-linear function of best fit to the data denoted by open circles and triangles, the same data used to generate the power function, was

$$R = A + B \log(C) \qquad (11)$$

where $A = 7.11 \pm 0.86$, $B = 0.59 \pm 0.087$, and C and R are as in Eq. **2**. The coefficient of determination was $r^2 = 0.83$ compared with 0.92 for the power function. This difference alone is not enough to favour use of the power function over the log-linear function, but there are other considerations which make the power function the regression equation of most utility (see Discussion).

Treatment of polarizations induced by control stimuli

In the past the antennal polarization elicited by a blank stimulus has been poorly understood and largely ignored. Typically, when an EAG occurs in response to a blank stimulus it is simply subtracted from the EAG elicited by the stimulus and the net is considered to be the "true" response. The results of our investigations of stimulus blanks suggest, however, that the blank must be treated differently.

When the SP1 procedure was used, the EAG resulting from a purged blank dispenser was highly variable and sometimes went positive in direction. When the SP2 procedure was used, however, the EAG from a purged blank dispenser was always negative, if still variable. When the blank was repeated at short intervals, the EAGs progressively diminished to zero. It could be argued at this point that the diminution of response could have resulted from receptor adaptation. However, several unreported experiments provided little to support such an argument, and, indeed, such adaptive effects have seldom been observed in recordings from individual receptor cells. In addition, when the carrier air was begun, a positive antennal polarization resulted that continued until the flow ceased (see Fig. 5).

To explain the findings from the SP1 and SP2 blanks, we hypothesized that the positive polarization was due to the cessation of the negative polarization that is elicited by humidity (Grant, 1970; Mayer, 1973b) and other odours present in the laboratory. This hypothesis was supported in other experiments that showed a decrease in the rate of spontaneous action potentials by pheromone-sensitive olfactory receptor neurones when pure dry air was constantly flushed over the antenna. Further, we suspected that air allowed to stand in the Teflon lines became contaminated with an olfactory stimulant (probably a Teflon plasticizer). Subsequently, it was shown that the longer air stands in the lines, the greater is the response to this source. Washing the lines with water or solvents did not eliminate this source of contamination, but purging the lines immediately before the stimulus did reduce the blank EAG to zero. Also, a small blank could be elicited by the residue from the cork stoppers used to seal the tubes. Consequently, we adopted the working hypothesis that blank polarizations are a result of three or more different stimuli: water vapour, extraneous room contaminants, and delivery line plasticizers.

DISCUSSION

The examination of processes comprising the EAG has led to new methodology and analyses that enhance its quantitative usefulness for studying insect olfaction. One of the enhancements is the elimination of the background by removing contamination from both the carrier and the stimulus airstream. For example, at the highest stimulus level the average amplitude obtained for *T. ni* by Mayer (1973b) was about 2 mv, Roelofs (1976) reported a 4-mv average. The average EAG response obtained with SP2 at the highest dose was 5.7 mv with a maximum above

7 mv. If these background sources are not controlled they can vary and the blank response must be remeasured frequently. Also, because the relationship between EAG and concentration is not linear, the presence of a large blank may reduce sensitivity. Because the background EAG is a result of a combination of extraneous sources, the common practice of subtracting the blank should be scrutinized carefully.

The stimulus confinement and resection procedures combined with detailed morphometric studies provided further confirmation that the EAG is a measure of summed contributions from individual olfactory sensilla. The introduction of the power function to analyze the response shows more clearly the interrelationships between the EAG and other measures of the olfactory response. In this concluding section we will consider the results concerning summation of sensillar contributions and the importance of the power function analysis in greater detail.

The basis of the EAG

Five years after his exploratory investigation of the EAG, Dietrich Schneider (1962) proposed that the EAG was the recording of the summed activity of many receptors, as affected by many outside factors. The results of both our stimulus confinement and progressive resection methods clearly showed that different levels of response from the antenna were obtained when different numbers of sensilla were stimulated (Figs 2, 3). The results thus support Schneider's hypothesis.

In further support, there are a few experiments utilizing other species where forms of stimulus confinement, as well as other methods, have been employed to record EAG responses from varying, although unknown numbers of sensilla. Nishino and Takayanagi (1979) confined the stimulus to various portions of the antenna of *Periplaneta americana* (L.). Their results demonstrated a differential antennal responsiveness that probably was a function of the number of sensilla stimulated. Both Payne *et al.* (1970) using *T. ni* and Behan and Schoonhoven (1978) studying *Pieris brassicae* (L.) obtained different responses from various regions of the antenna by situating the recording electrode at sites closer to the reference electrode at the base. Roelofs and Comeau (1971) also obtained a reduction of response by progressive resection of the antenna of *Argyrotaenia velutinana* (Walker). Nagai (1981) selectively stimulated the antenna of *Ostrinia nubilalis* (Hübner) and determined that the EAG was proportional to the length stimulated. He also proposed a modification of Schneider's hypothesis that, in addition to the contributions from generator potentials, the EAG also receives contributions from electrotonic potentials locally induced by the generator potentials. The generator potentials have a negative polarity in the distal direction of the antenna but the electrotonic potential spreads in both directions. Consequently, the net effect of any electrotonic potential on the results in our study is probably negligible.

Physiological significance of the power function

The finding that the EAG can be expressed as a power function of odourant concentration clarifies understanding of some of the interrelationships among the EAG, insect behavioural responses, and the responses of single olfactory neurones to pheromone. There are many examples in the literature where power functions have been applied to behaviour and single neuronal responses (Stevens, 1975), and at least one example from insects, that of *Bombyx mori* (Mankin and Mayer, 1983). It has been hypothesized that behavioural measures of stimulus intensity are power functions because they are correlated to the summed power function responses of single peripheral neurones through a CNS process called sensation (Stevens, 1975). If this concept can be applied to insects, then a power function relationship between behaviour and pheromone concentration indicates that the behavioural response may reflect the intensity of sensation in the CNS, which in turn would be a measure of the summed responses of pheromone receptor neurones. Because the code for intensity of sensation in the CNS is unknown, it would be difficult to demonstrate directly that such a process exists. Nevertheless, the concept of intensity of sensation is useful in modeling how different measures of peripheral detection and behavioural responses might be related.

Consider for example the following simplified model that interrelates the EAG, the single neurone response, and the behavioural response to a single pheromone component. Action potentials from single pheromone receptor neurones are transmitted to a locus in the antennal lobe of the deutocerebrum. The summed frequency of action potentials from all the receptor neurones at this locus is a measure of the intensity of sensation of pheromone. The intensity of the behavioural response is also a measure of the intensity of sensation.

The relationship of the EAG to behaviour in the model is more indirect than its relationship to the frequency of action potentials. The EAG is a measure of the summed generator potentials from which the action potentials are produced. Consequently, the EAG is correlated with intensity of sensation by its physiological relationship to the frequency of action potentials. The behaviour is correlated to the EAG only to the extent that the intensity of sensation is unmodified by the processes of discrimination and integration with other sensory modalities which also occur in the CNS.

The model provides a straightforward interpretation of the blank response and the correlation between the EAG and behaviour. In the model the EAG is the sum of contributions from several groups of sensory cells of a given response group. The contribution from each group is proportional to the mean response of each cell of that type times the number of cells in the group. To examine the responses of a particular group (or set of groups), e.g. the cells responsive to a particular pheromone component, it is preferable to avoid stimulating groups of other types. Elimination of this "blank" is even more important when one considers the nonlinearity of the EAG stimulus–response curve. Because the EAG apparently "saturates" at high stimulus levels, the additional response due to stimuli at elevated concentrations is not recordable.

In the model the correlation between the EAG and

behaviour is very complex because the EAG is an indirect measure of the input to the locus of the brain measuring stimulus intensity. The EAG provides no information about subsequent processing in the central nervous system. For some stereotyped behaviours that are responses to one or a small number of different stimuli, it may be possible, nevertheless, to correlate the magnitude of the EAG with the magnitude of the behavioural response. Such responses could include individual wing flutter or flight in appropriate environments or group averages of these behaviours. The behavioural response to complex mixtures of odours, however, is likely to correlate only weakly with the EAG magnitude because of the increased complexity of CNS integration and behavioural responses.

The utility of the model for suggesting future experiments with the EAG is exemplified by considering synergism at the periphery and the CNS. If synergism occurs at the periphery, the total antennal polarization at low stimulus levels should be a sum of the two separate responses expressed by the equation (see e.g. Berglund and Berglund, 1981):

$$R' = (R_1^2 + R_2^2 + 2R_1R_2B)^{1/2} \qquad \textbf{(13)}$$

where R_1 and R_2 are the power functions of Eq. **2** for the 2 different odourants, B is a regression constant that is proportional to the synergistic effect and R' is the resultant total EAG. If $B > 1$, synergism occurs, and if $B < 1$, inhibition or other competitive interactions occur. By contrast, if 2 odourants generate synergistic responses in the CNS then the antennal polarization should be the simple sum of the two power functions, i.e. $B = 1$ in Eq. **13**. It may be possible to determine the magnitude and the location of any synergism of response to two odourants by calculating the value of B in Eq. **13**.

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